BACTERIUM ANATUM, N. S., THE ETIOLOGIC FACTOR IN A WIDESPREAD DISEASE OF YOUNG DUCKLINGS KNOWN IN SOME PLACES AS "KEEL"

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In the spring of 1918, the attention of the senior author was called to a disease affecting very young ducklings on a commercial duck farm in Connecticut. The mortality in a lot of 3,000 ducklings, all hatched at about the same time, was almost 100 per cent. The symptoms were noticeable soon after hatching, and were as a rule as follows: The affected individuals appeared weak and sluggish, and remained close to the heating pipes. They were not easily aroused, and did not go in search of food, as did the others. They ate at times, however, and their crops were distended. One of the marked symptoms was intense thirst. After drinking, some of the ducklings drew themselves to full height, staggered for a few seconds, keeled over and, after one or two gasps, died — hence the name "keel" for the disease. Few of the ducklings died after they were from three to four weeks of age, the greatest mortality occurring within the first week or ten days. Some of the symptoms may be lacking or obscured in the affected broods. Perhaps the large majority of the victims of the disease die without warning to the observer, and are found dead under the hover or occasionally out in the open. This is seen particularly when the ducklings die very soon after leaving the shell.

Examination of the dead ducklings revealed no lesions or any other pathologic condition except, perhaps, paleness of the tissues as a whole and light body weight. By the ordinary streak method on nutrient agar an organism was isolated without difficulty from the liver, heart and lungs, which resembled B. pullorum, and at once appeared to be a member of the coli-typhi-paratyphi group. This organism was obtained from the tissues and blood of the first two ducklings examined, from others which were sent in from time to time from the same farm, and from the ovary and from an abdominal cyst of two ducks from a large commercial plant in Massachusetts.

A rather extensive inquiry into the methods of duck farming and the conditions affecting the industry in the North Atlantic states revealed some interesting facts. The magnitude of this enterprise is indeed surprising to one who

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is not already familiar with the situation. A duck farm near Wrentham, Mass. (Weber Brothers), lays the claim to having reared 75,000 ducks to marketing age in a single season. Only a short distance away on the same road there is another farm, with a capacity of from 40,000 to 50,000. Numerous other places in New England, especially in Massachusetts and Connecticut, are with more or less success carrying on business on a somewhat similar scale, as for example the Conway Farm in Newington. Long Island has for some years been known as a very important duck-raising section of the state of New York. The annual production on Long Island farms, large and small, can be safely estimated at a million dollars at least.

Raising of Peking ducks on a large scale has been a most profitable enterprise, at least up to the years 1917 and 1918. Few diseases seem to affect this breed, and little trouble has been encountered until recently in the rearing of ducklings, when conducted by competent and skilled persons. Furthermore, the demand for ducks of marketable age has been such as to stimulate still greater production.

During 1917 and 1918, particularly in the season of 1918, however, serious reverses were reported from various duck farms. The hatchibility of the eggs was poor, and in many instances the ducklings that left the shell died while very young, apparently from the disease discussed in this paper. While the season was too far advanced, when the inquiries were made, to obtain specimens for examination from the various farms, descriptions of the symptoms and conditions by the owners and managers of the affected stock were such as to lead to but one conclusion.

The losses from this disease in 1918 were very great. Not over 10,000 ducks were raised on a farm which had a previous record and a capacity of at least 40,000. The largest farm visited fell short of its 1917 production by fully 50,000, and the owner appeared very much alarmed over the outlook for the future when he stated that, whereas two years before the fields were white with ducks, they were now green with grass. Similar reports came from other sources. The conditions were in all probability greatly aggravated by the severe winter of 1917-18.

Several points of similarity between the disease which affected so many ducklings and bacillary white diarrhea of chicks impressed themselves on the senior author, who alone was concerned with the investigation at this time. The greatest mortality occurred in ducklings less than three weeks old, and few deaths resulted after the fourth week. The disturbance appeared to be of an intestinal nature, although little or no diarrheal condition could as a rule be observed. The ducklings became very weak and clung to the hover, indicating subnormal temperature. No pathologic changes were apparent on dissection, except, perhaps, anemia and light weight. The deaths in a brood did not occur in large numbers at any one time, but extended over a period of several days or even a few weeks. Finally, an organism was obtained from the affected ducklings examined which in many ways bore a close resemblance to Bacterium pullorum, the causative agent in bacillary white diarrhea.

On account of these points of resemblance between the two diseases, it appeared probable that they were similar in their origin and methods of transmission. While sufficient data have not thus far been gathered to show that "keel" has the same cycle of infection as bacillary white diarrhea of chicks, that is, that the organism causing it is transmitted through infected eggs of breeding ducks whose ovaries are infected, certain observations strongly point to such a relationship between parent and offspring.

EXAMINATION OF BREEDING STOCK

Late in July, 1918, eight breeding ducks on an infected farm were killed and the ovaries in particular subjected to a thorough pathologic and bacteriologic examination. The following is a brief record:

DUCK 1.—The ovary was normal and well developed.

DUCK 2.—The ovary was well developed, with many large and small ovules. There were also three abnormal cysts which had once been ova; one of the size of a pea, and dark in color; another of about the same size, but firm, angular and discolored. Contents were composed of dried yolk-like material surrounded by an amber-colored clear fluid. The third cyst was smaller, discolored and slightly angular. The contents were semisolid. All three of these ovarian cysts yielded a pure, abundant growth of an organism apparently identical with that isolated from the original ducklings on the first farm.

DUCK 3.—The ovary was normal with the exception of one ovum of hazelnut size which was almost black and of a rubber-like consistency. The culture test was negative.

DUCK 4.—The ovary was well developed with three very small, slightly discolored ovules. One was negative; the other two were too small to culture.

DUCK 5.—The ovary was normal, but undeveloped.

DUCK 6.—The ovary was normal but undeveloped.

DUCK 7.—The ovary was normal with the exception of two very small opaque ovules. There was an abdominal cyst, dark and angular, resembling typical ovarian cyst of bacillary white diarrhea. The cyst was suspended by a light cord from the omentum, and contained cheesy matter mingled with clear amber-colored fluid. Culture tests were positive. The organism was identical with that obtained from the ovary of duck 2, present in contents in large numbers and unassociated with other bacteria.

DUCK 8.—The ovary was well developed. There was one dark, bloody ovule. The culture test was negative.

The other organs and tissues of the ducks examined were apparently normal, and hence require no comments.

One of the eight ducks (No. 2) examined had a distinctly pathologic ovary very closely resembling the typical white diarrhea ovary of hens, and contained an organism indistinguishable from the bac-

terium which was isolated at the outset of the investigation from the blood and organs of ducklings affected with the "keel," though on a different farm. A second duck (No. 7) showed little abnormality of the ovary, but harbored a characteristic cyst in the peritoneal cavity which contained the same organism as did the ovary of duck 2.

Unfortunately, it was too late in the season to obtain on this farm young ducklings that were affected or had died of the disease under observation. The greatest mortality occurred very early in the season, and the rate decreased until July, when there were apparently very few sick ducklings, and comparatively few eggs were being hatched. The same decrease in morbidity and mortality was reported from the other farms which were investigated. Attempts to fully demonstrate transmission of the disease from the ovary through the egg to the offspring were not as successful, therefore, as they would have been had they been begun early in the spring of the year. Further investigations along this line will be carried on as soon as conditions are again favorable.

CHARACTERIZATION OF THE ORGANISM CAUSING THE DISEASE.— BACTERIUM ANATUM, NOV. SPEC.

In a preliminary report on the investigation (1919)[1] the name of the causative organism was given as Bacterium anatis, the word anatis being the genitive singular of the Latin *anas*, meaning duck. A survey of the literature showed that Cornil and Toupet[2] (1888) had applied this name to an organism which they isolated from diseased ducks, but which was indistinguishable from B. avisepticus, the organism of ordinary fowl cholera. The genitive plural has been substituted here for anatis, the name of the new micro-organism, therefore, being Bacterium anatum.

Five representative strains of the organism were used in all of the following characterization studies:

No. 1, isolated as 8C from one of a lot of ducklings received from C farm.

No. 2, isolated as cyst W from abdominal cyst of breeding duck on W farm.

No. 3, isolated as ovum W from ovarian cyst of breeding duck on W farm.

No. 4, isolated as IIC from second lot of ducklings received from C farm.

No. 5, isolated as VC from a third lot of ducklings received from C farm.

[1] Rettger, Leo F., and Scoville, Margaret M.: Bacterium anatis, Nov. Spec., an Organism of Economic Importance and a Member of the Paratyphoid Group of Bacteria. Paper presented before the Society of American Bacteriologists at the Annual Meeting, Baltimore, December, 1918.

[2] Cornil and Coupet: Sur une nouvelle maladie bactérienne du canard (cholera des canards), Compt. rend. de l'Acad. des Sciences de Paris, 1888, 106, p. 1737-50.

For purposes of convenience these different strains will be referred to as strains 1, 2, 3, 4 and 5.

It soon became apparent that the different strains revealed but very slight and insignificant differences among themselves, and that the organism, B. anatum, must be classed with the coli-typhi-dysenteriae group of bacteria, along the side of the well-known paratyphoid members. Hence, the investigation of the properties of B. anatum included a comparative study of this organism and B. typhi, B. paratyphosus A and B, B. enteritidis, B. pullorum (Rettger) and B. sanguinarium (Moore).[3]

MORPHOLOGY, STAINING PROPERTIES, ETC., OF BACTERIUM ANATUM

It is a short, rod-shaped, actively motile organism, in form resembling the shorter varieties of B. coli and B. enteritidis, rather than B. typhosus. It is actively motile, and possesses several peritrichiate flagella, although the exact number could not be definitely demonstrated. There are some variations in the length of the bacilli in the same culture medium. The average size may be stated approximately as 0.5 by 1 to 2 mikrons. It takes the ordinary stains readily, and is gram-negative.

The optimum temperature of B. anatum is 35-37 C., but it grows well at ordinary room temperature. It is a facultative anaerobe, having a very decided preference for atmospheric oxygen.

CULTURAL CHARACTERISTICS

Gelatin and Agar Plates.—The colonies have no characteristics that set them apart from the colonies of other members of the group. Growth is slightly heavier than that of B. typhosus, and less luxuriant than the colonies of B. coli.

Slant Agar.—Growth following inoculation with the needle by the single streak method is moderately abundant at the end of 24 hours at incubation temperature, and is indistinguishable from B. paratyphosus B and B. enteritidis. The growth becomes quite luxuriant and opaque after the first two days, and often shows more or less

[3] Smith, Theobald, and Ten Broeck, C.: Agglutination Affinities of a Pathogenic Bacillus from fowls (Bacterium sanguinarium, Moore) with the Typhoid Bacillus of Man, Jour. Med. Research, 1915, 31, p. 503-521.

Smith, Theobald, and Ten Broeck, C.: A Note on the Relation between B. pullorum (Rettger) and the Fowl Typhoid Bacillus (Moore). Idem, p. 547-555.

wrinkling eventually near the base. When the inoculation is made over the entire surface of the agar with a platinum loop, and with the blood from infected organs or with a light suspension of the organism in water or other liquid medium, discrete colonies are formed over the agar surface which in the early stages very closely resemble those of B. pullorum and which remain separate even after from 24 to 36 hours at incubation temperature. The older colonies are decidedly larger and more opaque than those of B. pullorum.

Gelatin Stab.—There is light growth along needle track, with little or no spreading on the surface. The gelatin is not liquefied.

Nutrient Broth.—Heavy clouding and surface pellicle occur in from 24 to 36 hours at 35-37 C. There is no appreciable odor.

Litmus Milk.—Faintly acid within 48 hours, but gradually changing to alkaline. Marked alkalinity at the end of 12 to 14 days. Reaction in litmus milk similar to that of strains of B. sanguinarium Moore and B. paratyphosus B employed in a series of comparative tests.

Potato.—The reaction on this medium differed more or less with the strains and with their viability. Strains 2 and 3 produced a scanty growth with no discoloration of the medium. Numbers 1, 4 and 5 developed a heavy, moist growth which in from 2 to 3 days became light brown, and brought about discoloration of the potato. After repeated transplantation on new medium, at intervals of 2 weeks, 5 was the only strain which discolored the potato.

No indol was produced in Dunham's peptone, nor in tryptophane broth, and all failed to give the Voges-Proskauer reaction.

Nitrates were reduced to nitrites by all five of the strains of B. anatum in 24 hours at 37 C.

All of the strains were able to utilize the nitrogen of ammonia, nitrate, urea, creatin and nucleic acid, but failed to attack uric acid.

ACTION ON CARBOHYDRATES AND OTHER FERMENTABLE SUBSTANCES

Gas production was determined in the double-barreled fermentation tube of Durham, the gas being measured by the Frost gasometer. Brom cresol purple was employed for the detection of acidity, only a P_H value of 5.6 or less being recorded. The cultures were incubated at 37 C.

The following fermentable substances were used; glucose, levulose, maltose, saccharose, lactose, galactose, inulin, dextrin, arabinose, rham-

nose, raffinose, xylose, inosit, adonitol, dulcitol, mannitol, salicin and glycerol. Standard nutrient bouillon was the medium employed for the ordinary fermentation tests. The test agents were as a rule one per cent. strength. Some of the rarer substances (inosit, adonitol and dulcitol) were used in 0.25 per cent.

All of the five strains of B. anatum gave the same reaction, with the following exceptions: Number 2 failed repeatedly to produce acid or gas in xylose, and number 3 gas in the same medium, while the others acidified the medium and produced 10 per cent. of gas. The reactions may be summarized briefly as follows:

Substances acted on, with acid production — glucose, levulose, maltose, galactose, dextrin, arabinose, rhamnose, inosit, xylose (except strain number 2), dulcitol, mannitol and glycerol.

Substances acted on, with gas formation, and the percentage of gas produced — glucose 40, levulose 25, maltose 10, galactose 35, dextrin 5-10, arabinose 10-15, rhamnose 10-15, inosit 10, xylose 10 (excepting strains number 2 and 3), mannitol 20, and dulcitol 35-40. Neither acid nor gas is formed from lactose, saccharose, inulin, raffinose, adonitol and salicin.

A comparison of these reactions with those obtained with B. typhosus and the common members of the paratyphoid group revealed a very close resemblance between B. anatum and B. paratyphi A and B, and B. enteritidis (see tables 1 and 2).

TABLE 1

ACID PRODUCTION BY BACTERIUM ANATUM AND RELATED ORGANISMS

Fermentable Substances	B. anatum Strains					B. typhosus	B. paratyphosus A	B. paratyphosus B	B. enteritidis	B. sanguinarium	B. pullorum
	1	2	3	4	5						
Glucose	+	+	+	+	+	+	+	+	+	+	+
Levulose	+	+	+	+	+	+	+	+	+	+	+
Lactose	0	0	0	0	0	0	0	0	0	0	0
Galactose	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	0
Saccharose	0	0	0	0	0	0	0	0	0	0	0
Arabinose	+	+	+	+	+	0	+	+	+	⊕	+
Rhamnose	+	+	+	+	+	0	+	+	+	0	+
Raffinose	0	0	0	0	0	0	0	0	0	0	0
Xylose	+	0	⊕	+	+	⊕	0	+	+	0	0
Dextrin	+	+	+	+	+	⊕	+	+	+	+	0
Inulin	0	0	0	0	0	0	0	0	0	0	0
Glycerol	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	+	0	0
Inosite	+	+	+	+	+	0	0	+	0	0	0
Dulcitol	+	+	+	+	+	0	+	+	+	+	0
Mannitol	+	+	+	+	+	+	+	+	+	+	+
Adonitol	0	0	0	0	0	0	0	0	0	0	0
Salicin	0	0	0	0	0	0	0	0	0	0	0

+ indicates acid production; 0 failure to produce gas; and ⊕ slight or doubtful reaction.

TABLE 2

GAS PRODUCTION BY BACTERIUM ANATUM AND RELATED ORGANISMS

Fermentable Substances	B. anatum Strains					B. typhosus	B. paratyphosus A	B. paratyphosus B	B. enteritidis	B. sanguinarium	B. pullorum
	1	2	3	4	5						
Glucose	40%	40%	40%	40%	40%	0	20%	35%	30%	0	25%
Levulose	25%	25%	25%	25%	25%	0	15%	30%	25%	0	15%
Lactose	0	0	0	0	0	0	0	0	0	0	0
Galactose	35%	35%	35%	35%	35%	0	20%	25-35%	25%	0	5-10%
Maltose	10%	10%	10%	10%	10%	0	30%	30%	30%	0	0
Saccharose	0	0	0	0	0	0	0	0	0	0	0
Arabinose	10-15 %	10-15 %	10-15 %	10-15 %	10-15 %	0	10%	20%	20%	0	—
Rhamnose	10%	15%	10%	10%	15%	0	10%	—	—	0	—
Raffinose	0	0	0	0	0	0	0	0	0	0	0
Xylose	10%	0·	0	10%	10%	0	0	20%	40%	0	0
Dextrin	8%	8%	10%	10%	10%	0	5%	5%	5%	0	0
Inulin	0	0	0	0	0	0	0	0	0	0	0
Inosite	10%	10%	10%	10%	10%	0	0	10%	0	0	0
Glycerol	0	0	0	0	0	0	0	0	0	0	0
Dulcitol	38%	38%	40%	40%	40%	0	30%	35%	30%	0	0
Mannitol	20%	20%	20%	20%	20%	0	30%	30-35%	35%	0	5-10%
Adonitol	0	0	0	0	0	0	0	0	0	0	0
Salicin	0	0	0	0	0	0	0	0	0	0	0

HYDROGEN-ION CONCENTRATION OF BACTERIUM ANATUM AND RELATED ORGANISMS

The hydrogen-ion concentration was determined by the colorimetric method of Clark and Lubs. Both the peptone and the synthetic phthalate mediums of these authors were employed. The cultures were incubated at 37 C. for 5 days. Methyl red was used as the indicator, 5 drops of this reagent being added to 5 c c of the culture.

TABLE 3

RESULT OF TESTS

Organisms	Peptone Medium P_H =	Synthetic Medium P_H =
B. anatum 1	4.9	4.6
B. anatum 2	4.8	4.6
B. anatum 3	4.9	4.6
B. anatum 4	4.8, 4.9	4.6
B. anatum 5	4.9	4.6
B. typhi	5.2	5.4
B. paratyphi A	5.0, 4.9	4.9
B. paratyphi B	4.9	4.8
B. pullorum	4.9	5.1
B. sanguinarium	5.2	5.0

The uniformity of the P_H values of the different strains of B. anatum in both mediums, with a slightly higher hydrogen-ion concentration in the synthetic than in the peptone medium, is of some interest. In the peptone medium the figures for all of the organisms employed

vary only within a few tenths, whereas the figures of the second column show somewhat greater irregularity among the different species.

AGGLUTINATION STUDIES

Agglutination experiments were conducted with the serum of rabbits which were immunized against one or another of the following organisms: B. anatum, 1, B. typhosus, B. paratyphosus A, B. paratyphosus B, B. pullorum, and B. sanguinarium.*

TABLE 4

AGGLUTINATION EXPERIMENTS WITH THE SERUM OF RABBIT IMMUNIZED AGAINST B. ANATUM, 1

Antigen	1:100	1:200	1:400	1:500	1:800	1:1,000	1:2,000	1:4,000
B. anatum 1	++++	++++	++++	+++	+++	+++	+++	+++
B. anatum 2	++++	++++	++++	++++	++++	++++	++++	++++
B. anatum 3	++++	++++	++++	++++	+++	+++	+++	+++
B. anatum 4	++++	++++	++++	+++	+++	++	++	+
B. anatum 5	++++	++++	++++	+++	+++	+++	+++	?
B. typhosus 3, Am. M.	++++	++	0	0	0	0	0	0
B. paratyphosus A, 228K	0	0	0	0	0	0	0	0
B. paratyphosus A, 258K	0	0	0	0	0	0	0	0
B. paratyphosus A, 287K	0	0	0	0	0	0	0	0
B. paratyphosus B, 225K	+++	+++	+++	+++	++	++	++	+
B. paratyphosus B, 234K	++++	+++	++	++	++	++	0	0
B. paratyphosus B, 232K	+++	++	++	+	+	+	0	0
B. sanguinarium, C.	0	0	0	0	0	0	0	0
B. pullorum, B'16	0	0	0	0	0	0	0	0

++++ indicates complete agglutination; +++, almost complete; ++, partial; +, slight; and 0, no agglutination.

TABLE 5

SERUM OF RABBIT IMMUNIZED AGAINST B. TYPHOSUS, 607, AM. M.

Antigen	1:100	1:200	1:400	1:500	1:800	1:1,000	1:2,000	1:4,000
B. anatum 1	0	0	0	0	0	0	0	0
B. anatum 2	0	0	0	0	0	0	0	0
B. anatum 3	0	0	0	0	0	0	0	0
B. anatum 4	0	0	0	0	0	0	0	0
B. anatum 5	0	0	0	0	0	0	0	0
B. typhosus 607, Am. M.	++++	++++	++++	++++	+++	+++	+	0
B. typhosus 3, Am. M.	++++	++++	+++	+++	++	++	+	0
B. paratyphosus A, 228K	0	0	0	0	0	0	0	0
B. paratyphosus A, 258K	0	0	0	0	0	0	0	0
B. paratyphosus A, 287K	0	0	0	0	0	0	0	0
B. paratyphosus B, 234K	0	0	0	0	0	0	0	0
B. paratyphosus B, 225K	0	0	0	0	0	0	0	0
B. paratyphosus B, 232K	0	0	0	0	0	0	0	0
B. sanguinarium, C.	0	0	0	0	0	0	0	0
B. pullorum, B'16	0	0	0	0	0	0	0	0

Slant agar cultures grown at 37 C. for 24 hours were employed. The growths were washed off with salt solution, and suspensions of definite density, in so far as these could be obtained by the nephelometer method, were administered subcutaneously. Four injections of heated vaccines, in doses of 0.5, 1.0,

* Since the rabbit which was inoculated with B. sanguinarium did not furnish a serum of marked agglutinating properties, this serum is not included in the following tables.

1.5 and 2.0 c c were followed by 0.5 c c of a living suspension, all at 4-5 day intervals. On account of the weak agglutination properties of the blood serums further subcutaneous injections of 0.25, 0.75, 1.25, and 2.0 c c of the suspensions were made. The final agglutination tests were conducted on blood samples drawn on the tenth day after the last inoculation.

In the agglutination tests the serum dilutions varied from 1 : 100 to 1 : 4,000. The antigens were prepared by washing off 24-hour agar cultures of the different organisms employed (B. anatum, B. typhosus, B. paratyphosus A, B. para-

TABLE 6

SERUM OF RABBIT IMMUNIZED AGAINST B. PARATYPHOSUS A, 228K

Antigen	1:100	1:200	1:400	1:500	1:800	1:1,000	1:2,000	1:4,000
B. anatum 1	0	0	0	0	0	0	0	0
B. typhosus, 607	0	0	0	0	0	0	0	0
B. paratyphosus A, 228K	++++	++++	++++	++++	++++	+++	+++	+++
B. paratyphosus B, 232K	0	0	0	0	0	0	0	0
B. sanguinarium	0	0	0	0	0	0	0	0
B. pullorum	0	0	0	0	0	0	0	0

TABLE 7

SERUM OF RABBIT IMMUNIZED AGAINST B. PARATYPHOSUS B, 232K

Antigen	1:100	1:200	1:400	1:500	1:800	1:1,000	1:2,000	1:4,000
B. anatum 1	+++	+++	++	++	+	+	+	+
B. anatum 2	0	0	0	0	0	0	0	0
B. anatum 3	++	++	0	0	0	0	0	0
B. anatum 4	++	++	++	+	+	+	0	0
B. anatum 5	+++	++	++	+	+	0	0	0
B. typhosus, 3	+?	0	0	0	0	0	0	0
B. typhosus, 607	++	0	0	0	0	0	0	0
B. paratyphosus A, 228	0	0	0	0	0	0	0	0
B. paratyphosus A, 258	0	0	0	0	0	0	0	0
B. paratyphosus A, 287	0	0	0	0	0	0	0	0
B. paratyphosus B, 225	++++	++++	++++	++++	+++	+++	+++	++
B. paratyphosus B, 232	++++	++++	++++	++++	+++	+++	++	+
B. paratyphosus B, 234	++++	++++	++++	++++	+++	+++	++	+
B. sanguinarium	0	0	0	0	0	0	0	0
B. pullorum	0	0	0	0	0	0	0	0

TABLE 8

SERUM OF RARRIT IMMUNIZED AGAINST B. PULLORUM

Antigen	1:100	1:200	1:400	1:500	1:800	1:1,000	1:2,000	1:4,000
B. anatum 1	0	0	0	0	0	0	0	0
B. anatum 2	0	0	0	0	0	0	0	0
B. anatum 3	0	0	0	0	0	0	0	0
B. anatum 4	0	0	0	0	0	0	0	0
B. anatum 5	0	0	0	0	0	0	0	0
B. typhosus, 3	++++	++++	+++	+++	++	0	0	0
B. typhosus, 607	++++	++++	+++	+	0	0	0	0
B. paratyphosus A, 228	0	0	0	0	0	0	0	0
B. paratyphosus A, 258	0	0	0	0	0	0	0	0
B. paratyphosus A, 287	0	0	0	0	0	0	0	0
B. paratyphosus B, 225	0	0	0	0	0	0	0	0
B. paratyphosus B, 232	0	0	0	0	0	0	0	0
B. paratyphosus B, 234	0	0	0	0	0	0	0	0
B. sanguinarium	++++	+++	++	+	+	+	0	0
B. pullorum	++++	++++	++	+	0	0	0	0

typhosus B, B. sanguinarium and B. pullorum) with carbolized salt solution. They were made to match in turbidity with 0.75 of the McFarland nephelometer scale.

The close relationship of B. anatum to B. paratyphosus B is again brought out vividly. Each of the three strains of B. paratyphosus B. employed was agglutinated in a dilution of at least 1 : 1,000 when tested with the serum of a rabbit which was immunized against B. anatum. Furthermore, four of the five strains of B. anatum reacted with the serum of the paratyphosus B rabbit, one in a dilution of 1 : 4,000. However, agglutination was in but one instance complete. B. anatum 2 failed to react. Excepting a reaction of B. anatum serum with B. typhosus in dilutions of 1 : 100 and 1 : 200, and a partial reaction in 1 : 100 of B. typhosus to the B. paratyphosus B serum, no other cross-agglutinations were observed.

All five of the B. anatum strains were agglutinated in high dilution by the serum of the rabbit immunized against B. anatum 1, strain 2 being completely clumped in a dilution of 1 : 4,000. The failure of B. pullorum and B. sanguinarium to react with the B. anatum serum is of particular interest, and further strengthens the view that B. anatum and B. pullorum are not only not identical, but that their relationship with each other is rather remote.

The cross agglutination of B typhosus and B. sanguinarium with the B. pullorum serum is in accord with the observations of Smith and Ten Broeck (1915) who demonstrated a very close relationship between these three organisms. The serum of the B. typhosus rabbit failed, however, to agglutinate B. sanguinarium and B. pullorum.

PATHOGENICITY OF B. ANATUM

Four chicks about three weeks old were inoculated with 0.25 c c of a suspension of B. anatum prepared by washing off a 24-hour slant agar culture with 20 c c of salt solution. The inoculations were made under the skin of the breast.

Within five hours after the injections, two of the chicks appeared decidedly lame. On the morning of the following day these chicks were found dead, and the other two unable to walk. Five hours later a third died. The remaining chick succumbed late in the afternoon two days later. All of the chicks died within 53 hours after inoculation. The quick response to the small doses administered must be regarded as an indication of a high degree of pathogenicity of the organism. Results obtained in later experiments were, however, not so striking; but this may at least in part be accounted for by the brief interval (2-3 weeks) which had elapsed after the isolation of the organism injected in the first series of experiments on young chicks and the time of later inoculations.

Aside from reddening of the area immediately surrounding the point of inoculation, no abnormal condition of the chicks as a whole or of the internal organs was apparent. On postmortem examination, B. anatum was recovered.

At the same time that the four chicks mentioned above were inoculated, a half-grown rabbit and an adult guinea-pig received a subcutaneous injection of 1.0 c c of the same bacterial suspension.

Five days after the injections, a large swelling was observed on the rabbit at the site of inoculation. This grew to about the size of a walnut, and was firm to the touch. It soon broke and yielded a fluid resembling ordinary pus. The abscess was treated daily with disinfectant, but persisted for several days. Healing was very slow. A similar reaction occurred on the guinea-pig, though the swelling was not as large and prominent as on the rabbit. Attempts to isolate B. anatum from the pus of the abscesses were unsuccessful.

Three chicks, 11 days old, and a fourth, 10 weeks old, were inoculated in the same manner as the preceding. The smaller chicks received from 0.25 to 0.5 c c of the bacterial suspension, and the older chick 1.0 c c.

Within from 24 to 36 hours the three small chicks showed indications of bodily disturbance. They were quiet and of subnormal temperature. They ate freely, and the crops were distended; they were thirsty also. The condition grew worse gradually. Four days after injection they were very weak and listless, their eyes closed, and they huddled together for warmth. One of the chicks died on the following day. Two days later a second died, and nine days after inoculation the third was found dead.

The tissues and organs of the three chicks showed no abnormal appearances except a slight swelling and the presence of a small amount of fibrous exudate at the site of inoculation in two of the chicks. B. anatum was recovered from the liver and heart blood in large numbers and unassociated with other organisms.

Fourteen ducklings, two days old, were divided into three groups of 4, 5 and 5. Four ducklings in each lot were given a subcutaneous injection of a salt solution suspension of B. anatum grown on slant agar for 24 hours. Lot 1 (4 in the group, and no control) received strain 3; 2, strain 1, and lot 3 strain 4. Lots 2 and 3 also contained an uninoculated control. The amount of bacterial suspension injected was 0.25 c c.

During the first 48 hours there was no visible change in any of the ducklings. On the third day a duckling was found dead in pen 2, and a little later on the same day one in pen 1. On the following day two others died in lot 1. On the fourth day but one remained alive in this pen. A second duckling was found dead in pen 2 on the fourth day after inoculation. On the same day the control bird of this lot was found dead in the feed dish which had become filled with water during a rain storm. The duckling had the appearances of being trampled on by the hen which was brooding them.

Lot 3 suffered its first loss on the fourth day. This duckling appeared abnormal, and the same staggering was observed as was seen on the farm where the first inquiry was made into the disease. The last survivor of lot 3 became lame on the third day after inoculation. A nodular swelling was observed at the site of inoculation which resembled those seen in the rabbit and guinea-pig. The swelling appeared to be painful, and the duckling moved about very little. It remained alive until 11 days after the injection. Postmortem examination revealed an abundance of fibrous exudate at the inoculation site, and for some distance over the right side of the breast and on the right thigh. The internal organs appeared normal. B. anatum was recovered from the liver and spleen in large numbers.

At the end of 11 days there were left of the original ducklings, 2 in pen 1, 3 in pen 2, and none in pen 3.

Culture tests on all of the dead ducklings yielded a pure growth of B. anatum from the blood of the internal organs in every instance except in the first victim and in the control. No pathologic changes could be observed except a swelling and accumulation of a fibrous exudate in the inoculation area on three or four of the ducklings.

SUMMARY

B. anatum, N. S., was isolated from the internal organs of young ducklings that died of a disease in some respects resembling bacillary white diarrhea of chicks and known in some sections as "keel." The disease is of apparently wide distribution, and the mortality may be very high.

The organism was obtained in pure culture also from ovarian cysts and from an abdominal cyst of breeding ducks on a large commercial duck farm which was very seriously affected by the duckling disease. It appears quite probable, therefore, that the disease is transmitted from the breeders through infected eggs in the same way as bacillary white diarrhea has been demonstrated to have its source in the ovaries of infected hens. Proof of such relationship in the duckling disease is, however, still lacking, owing to our inability to procure the necessary materials for a more extensive study of this point. It is planned to resume this phase of the investigation with the appearance of the next breeding season.

While B. anatum resembles B. pullorum in several particulars, it is more closely related, in so far as morphology, cultural, and fermentation properties are concerned, to B. paratyphosus A and B, and to B. enteritidis. Its agglutination reactions link it most closely with B. paratyphosus B; also its alkalinizing action in milk. Of the different organisms which have been used in the comparative study, namely, B. typhosus, B. paratyphosus A and B, B. enteritidis, B. pullorum and B. sanguinarium, B. anatum resembles B. typhosus least, and B. paratyphosus B most.